FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Mini Kit

User Manual

Cat. No.: FABGK 001 (50 Preps)
FABGK 001-1 (100 Preps)
FABGK 001-2 (300 Preps)

For Research Use Only

v.1002

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Introduction

FavorPrep™ Genomic DNA Extraction Mini Kit is an excellent tool offering a speedy and economic method to purify total DNA (e.g. genomic, mitochondrial and viral DNA) from whole blood (fresh or frozen), plasma, serum, buffy coat, body fluids, lymphocytes and cultured cells. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K, then binds DNA to silica-based membranes, washes DNA with ethanol-contained Wash Buffer and then elutes purified DNA by low salt Elution Buffer or ddH_2O. Compare with other harmful and time-consuming procedures, such as phenol/chloroform extraction and ethanol precipitation, FavorPrep™ shortens the handling time within 1 hour. The size of purified DNA is up to 50 Kb (predominantly 20 - 30 Kb). After using FavorPrep™ Genomic DNA Extraction Mini Kit, the high quality total DNA can be used directly for the downstream applications.

Specification

Sampling: up to 200 µl of whole blood (with anti-coagulant), plasma, serum, buffy coat, or body fluids; up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS.

Yield: about 5 µg of total DNA from 200 µl of human whole blood; up to 50 µg of total DNA, depends on the sample types and the number of cells in the sample.

Handling time: within 1 hour, depends on the sample types.

Kit Contents

	FABGK001 (50preps)	FABGK001-1 (100preps)	FABGK001-2 (300preps)
FABG Buffer	15 ml	30 ml	70 ml
W1 Buffer*	22 ml	44 ml	124 ml
Wash Buffer**	10 ml	20 ml	50 ml
Elution Buffer	15 ml	30 ml	30 ml x 3
Proteinase K***	11 mg	11 mg x 2	11 mg x 6
FABG Mini Column	50 pcs	100 pcs	300 pcs
Collection Tube	100 pcs	200 pcs	600 pcs
Elution Tube	50 pcs	100 pcs	300 pcs

*Add 8 / 16 / 45 ml ethanol (96~100%) to W1 Buffer when first open.

**Add 40 /80 / 200 ml ethanol (96~100%) to Wash Buffer when first open.

***Add 1.1 ml sterile ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution.

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

2. Add 1.1 ml sterile ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4°C**.

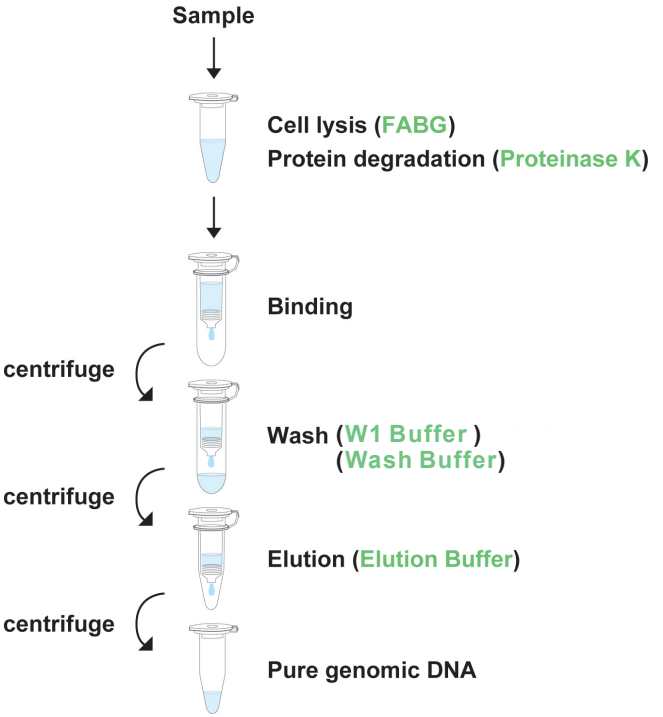
3. For FABGK 001(50preps), add 8ml ethanol (96~100%) to W1 Buffer when first open. For FABGK 001-1 (100presp), add 16 ml ethanol (96~100%) to W1 Buffer when first open. For FABGK 001-2 (300presp), add 45 ml ethanol (96~100%) to W1 Buffer when first open.

4. For FABGK001 (50preps), add 40ml ethanol (96~100%) to Wash Buffer when first open. For FABGK 001-1 (100preps), add 80 ml ethanol (96~100%) to Wash Buffer when first open. For FABGK 001-2 (300preps), add 200 ml ethanol (96~100%) to Wash Buffer when first open.

5. Preheat a dry bath or a water bath to 60°C before the operation.

6. All centrifuge steps are done at full speed (14,000 rpm or 10,000 xg) in a microcentrifuge.

Brief Procedure



General Protocol

Please Read Important Notes Before Starting The Following Steps.
HINTP: Preheat a 60°C dry bath or water bath for step 4.

1.Transfer up to 200 µl sample (whole blood, buffy coat) to a micropcentrifuge tube (not provided).

・If the sample volume is less than 200 µl, add the appropriate volume of PBS.

2. (Optional): If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 minutes at room temperature.

3. Add 20 µl Proteinase K and 200 µl FABG Buffer to the sample. Mix thoroughly by pulse-vortexing.

・Do not add Proteinase K directly to FABG Buffer.

4. Incubate at 60°C for 15 minutes to lyse the sample. During incubation, vortex the sample every 3 - 5 minutes.

5. Briefly spin the tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96~100%) to the sample. Mix thoroughly by pulse-vortexing for 30 seconds.

7. Briefly spin the tube to remove drops from the inside of the lid.

8. Place a FABG Column to a collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 1 minute and discard the flow-through then place FABG Column to a new Collection tube.

9. Immediately, Wash FABG Column with 500 µl W1 Buffer by centrifugefor 1 minute then discard the flow-through.

- Make sure that ethanol has been added into W1 Buffer when first open.

10. Wash FABG Column with 750 µl Wash Buffer by centrifuge for 1 minute then discard the flow-through.

- Make sure that ethanol has been added into Wash Buffer when first open.

11. Centrifuge for an additional 3 min to dry the column.

- **Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

12. Place FABG Column to Elution Tube.

13. Add 100~200 µl of Elution Buffer or ddH_2O (pH 7.5~9.0) to the membrane center of FABG Column. Stand FAGB Column for 3 min.

- **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
- Standard volume for elution is 200 µl, If sample has low number of cells, reduce the elution volume (50µl - 150µl) to increase DNA concert ration.

14. Centrifuge for 2 min to elute the DNA .

15. Store the DNA fragment at 4°C or -20°C.

Special Protocol

For cultured Cells

1. Harvest Cells

a. Cells grown in suspension

i. Transfer the appropriate number of cell (up to 5 x 10^6) to a 1.5ml microcentrifuge tube (not provided).

ii. Centrifuge at 300 x g for 5 minutes.

iii. Remove the supernatant carefully and completely.

b. Cells grown in monolayer

i. Detach cells from the dish or flask by trypsinization or using a cell scraper.

ii. Transfer the appropriate number of cell (up to 5 x 10^6) to a 1.5ml microcentrifuge tube (not provided).

iii. Centrifuge at 300 x g for 5 minutes.

iv. Remove the supernatant carefully and completely.

2. Resuspend cell pellet in PBS to a final volume of 200µl.

3. Follow the General Protocol starting from step 2.

Preparation of buffy coat

Centrifuge whole blood at 3,300xg for 10 minutes at room temperature and you will get three different fractions: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; the bottom layer contains concentrated erythrocytes. Process the General Protocol from Step 1 for buffy coat. Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.

Trouble Shooting

Problem	Possible Reasons/ Solution
Low or no yield of genomic DNA	**1. Low amount of cells in the sample** ・Concentrate a larger volume of a new sample to 200 µl. If the sample is whole blood, prepare buffy coat (refer to Special Protocol on page 7).
	2. Poor cell lysis **A. it is because of insufficient Proteinase K activity** ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored proteinase K stock solution. **B. it is because of insufficient mixing with FABG Buffer** ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and thoroughly by pulse-vortexing. **C. It is because of insufficient incubation time.** ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	3. Ethanol is not added into the lysate before transferring sample mixture into FABG Column ・Repeat the extraction procedure with a new sample.
	4. Incorrect preparation of Wash Buffer **A. Ethanol is not added into Wash Buffer when first open.** ・Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Trouble Shooting

Problem	Possible Reasons/ Solution
Low or no yield of genomic DNA	**B. The volume or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample. **5. Elution of genomic DNA is not efficient** **A. PH of water (ddH_2O) for elution is acidic** ・Make sure the pH of ddH_2O is between 7.5~9.0. ・Use Elution Buffer (provided) for elution. **B. Elution Buffer or ddH_2O is not completely absorbed by column membrane** ・After Elution Buffer or ddH_2O is added, stand the FABG Column for 5 minutes before centrifugation.
Brown residues remain on the column membrane after washing	**1. Poor Cell Lysis** **A. It is because of insufficient Proteinase K activity** ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution. ・Do not add Proteinase K stock directly to FABG Buffer. **B. It is because of insufficient mixing with FABG Buffer** ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and thoroughly by pulse-vortexing. **C. Poor cell lysis because of insufficient incubation time** ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.

Trouble Shooting

Problem	Possible Reasons/ Solution
Brown residues remain on the column membrane after washing	**2. Ethanol is not added into the lysate before transferring sample mixture into FABG Column** ・Repeat the extraction procedure with a new sample.
	3. Incorrect preparation of Wash Buffer **A. Ethanol is not added into Wash Buffer when first open** ・Make sure that the correct volumnes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample. **B. The volumn or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumnes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.
Column is clogged	**1. Blood sample contains clots** ・Repeat the extraction procedure with a new sample. Mix the blood sample well with anticoagulant to prevent formation of blood clots. **2. Sample is too viscous** ・Reduce the sample volume. **3. Insufficient activity of Proteinase K** ・Use a fresh or well-stored Proteinase K Stock solution.. ・Repeat the extraction procedure with a new sample. Do not add Proteinase K Into FABG Buffer directly.

Trouble Shooting

Problem	Possible Reasons/ Solution
A_{280}/A_{280} ratio of eluted DNA is low	**Poor cells lysis**
	1. Poor cell lysis because of insufficient Proteinase K activity ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution. ・Do not add Proteinase K directly to FABG Buffer.
	2. Poor cell lysis because of insufficient mixing with FABG Buffer ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and throughly by pulse-vortexing.
	3. Poor cell lysis because of insufficient incubation time ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	Ethanol is not added into the lysate before transferring sample mixture into FABG Mini Column ・Repeat the extraction procedure with a new sample.
	Incorrect preparation of Wash Buffer
	4. Ethanol is not added into Wash Buffer when first open ・Make sure that the correct volumes of ethanol (96 - 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Trouble Shooting

Problem	Possible Reasons/ Solution
A_{280}/A_{280} ratio of eluted DNA is low	**5. The volume or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumes of ethanol (96 - 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.
	6. Genomic DNA is contaminated ・Do not wet the rim of the column during sample and buffer loading.
A_{280}/A_{280} ratio of eluted DNA is high	**1. A lot of residual RNA in eluted DNA** ・Follow the General Protocol step 2 to remove RNA.
	2. FABG Buffer added to the sample before adding RNase A ・Make sure that Rnase A has been added to the sample before adding FABG Buffer when using optional RNase step.
Degradation of eluted DNA	**1. Sample is old** ・Always use fresh or well-stored sample for genomic DNA extraction.
	2. Buffer for gel electrophoresis contaminated with DNase ・Use fresh running buffer for gel electrophoresis.

Storage Conditions

FavorPrep™ Genomic DNA Extraction Mini Kit except Proteinase K can be stored at room temperature 15 - 25°C for up to 1 year. Proteinase K powder can be stored dry at room temperature for up to 6 months. For storage longer than 6 months Proteinase K powder should be stored dry at 2-8°C. Proteinase K stock solution is stable for 2 months when stored at 2-8°C. Storage at -20°C will prolong its life, but repeated freezing and thawing should be avoided.

FavorPrep™ Genomic DNA Mini Kit (Blood/Cultured Cell)

User Manual

Cat. No.: FABGK 100 (100 Preps)
FABGK 300 (300 Preps)

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Kit Contents

Cat. No. / preps	FABGK 100 (100 Preps)	FABGK 300 (300 Preps)
RBC Lysis Buffer	135 ml	405 ml
FATG Buffer	30 ml	75 ml
FABG Buffer	40 ml	100 ml
W1 Buffer	45 ml	130 ml
Wash Buffer* (concentrated)	25 ml	50 ml
Elution Buffer	30 ml	75 ml
FABG Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

*Add 100 ml / 200 ml of ethanol (96~100%) to Wash Buffer when first open.

Specification

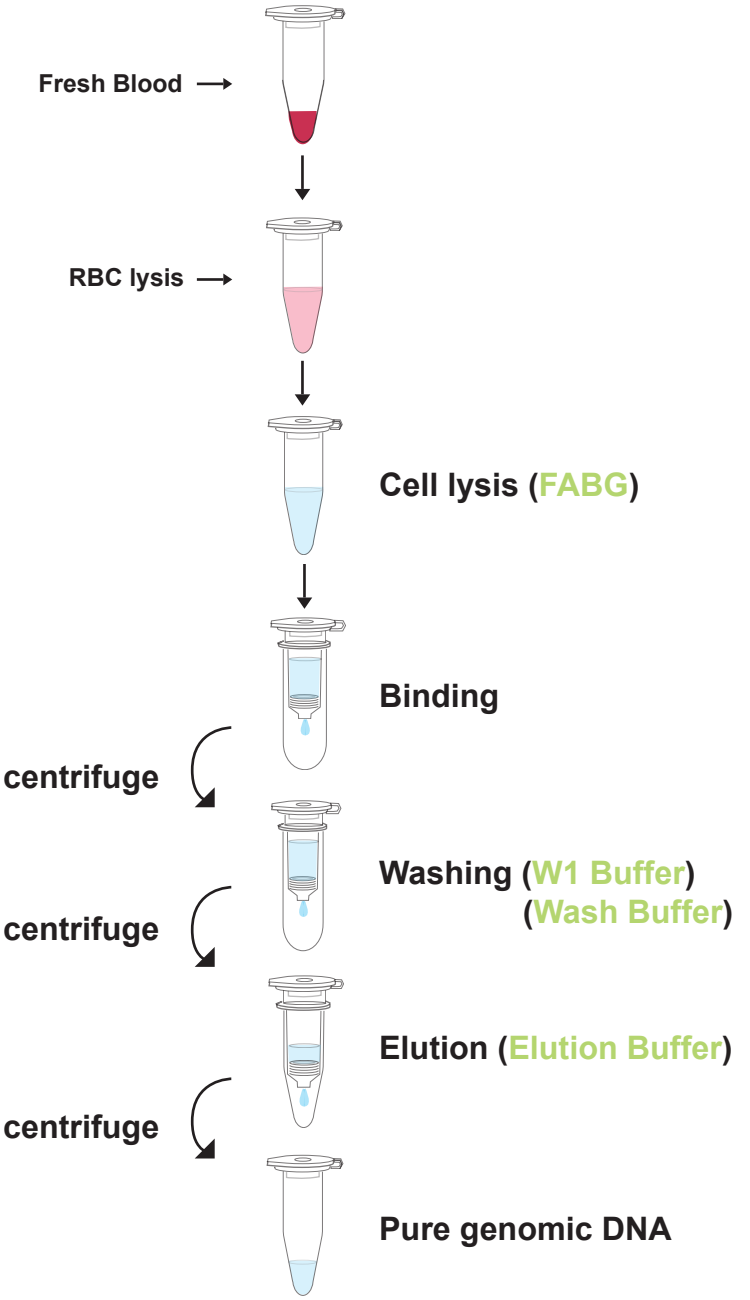
Sample Size: Up to 300 µl of Whole blood
Up to 200 µl of frozen blood
Up to 200 µl of buffy coat
Up to 1 x 10^7 of Cultured animal cells
Up to 1 x 10^9 of Cultured bacterial cells
Up to 5 x 10^7 of Fungus cells
Average Yield: about 50 µg
Format: spin column
Handling time: within 60 minutes

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

2. For Cat. No. FABGK 100, add 100 ml of ethanol (96~100%) to Wash Buffer when first open. For Cat. No. FABGK300, add 200 ml of ethanol (96~100%) to Wash Buffer when first open.

Brief Procedure



Genernal Protocol: (For Fresh Blood)

Please Read Important Notes Before Starting The Following Steps.

Step 1 – RBC Lysis

1. **Collect fresh human blood in an anticoagulant-treat collection tube.**

2. **Transfer up to 300µl fresh blood to a 1.5ml microcentrifuge tube (not provided). If the sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube.**

3. **Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.**

4. **Incubate at room temperature for 10 minutes.**

5. **Centrifuge at 3,000 x g for 5 minutes and completely remove the supernatant.**

6. **Resuspend the pellet with 100 µl of RBC Lysis Buffer.**

Step 2 – Cell Lysis

7. **Add 200 µl of FABG Buffer and mix by vortex.**

8. **Incubate for 10 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.**

9. **Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.**

10. (Optional Step)**: If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.**

Step 3 – Binding

11. **Add 200µl ethanol (96~100%) to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate.)**

12. **Place a FABG Column to a 2ml collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 5 minute at full speed (14,000 rpm or 10,000 x g) and discard the 2ml collection tube. Place the FABG Column in a new 2ml Collection tube.**

Step 4 – Washing

13. **Wash FABG Column with 400µl W1 Buffer. Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.**

14. **Place the FABG Column back in the 2ml Collection tube. Wash FABG Column with 600µl Wash Buffer (ethanol added). Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.**
    --Make sure that ethanol has been added into Wash Buffer when first open.

15. **Place the FABG Column back in the 2ml Collection tube. Centrifuge for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column.**
    --Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step 5 – Elution

16. **Place the dry FABG Column to a new 1.5ml microcentrifuge tube.**

17. **Add 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column. Stand FAGB Column for 3~5 min or until the buffer is absorbed by the membrane.**
    --Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely.

18. **Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) to elute the DNA .**
    --Standard volume for elution is 100 **µ**l. If sample has low number of cells, reduce the elution volume (30 **µ**l - 50 **µ**l) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 **µ**l.

Step Final - Pure DNA

19. **Store the DNA fragment at 4ºC or -20ºC.**

Special Protocol: (For Frozen Blood)

Step 1-Sample Preparation

1. **Transfer up to 200µl blood to a 1.5ml microcentrifuge tube (not provided). If the sample volume is less than 200µl, add the appropriate volume of PBS.**

2. **Add 30µl Proteinase K (10 mg/ml) to the sample and briefly mix. Then incubate for 15 minutes at 60°C.**

Step 2 –Cell Lysis

3. **Add 200µl FABG Buffer to the sample and mix by vortex.**

4. **Incubate in a 70°C water bath for 15 minutes to lyse the sample. During incubation, invert the sample every 3 minutes.**

5. **Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.**

6. (Optional Step)**: If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.**

7. **Follow the General Protocol starting from Step 3 (Binding).**

Special Protocol: (For Buffy Coat)

Step 1-Sample Preparation

Centrifuge whole blood at 3,300xg for 10 minutes at room temperature and you will get three different fractions: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; the bottom layer contains concentrated erythrocytes. Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.

Step 2 – RBC Lysis

1. **Transfer up to 200µl buffy coat to a 1.5ml microcentrifuge tube (not provided).**
2. **Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion.**
3. **Incubate at room temperature for 10 minutes. During incubation, invert the tube every 3 minutes.**
4. **Centrifuge for 1 minutes at full speed (14,000 rpm or 10,000 x g) and completely remove the supernatant.**
5. **Resuspend the pellet with 500 µl of RBC Lysis Buffer. Then centrifuge for 1 minutes at full speed (14,000 rpm or 10,000 x g) and completely remove the supernatant.**
6. **Resuspend the pellet with 200 µl of RBC Lysis Buffer. (Mix the tube by vortex only. Be sure the pellet is completely resuspended or the column would be barred when processing binding step.)**

Step 3 – Cell Lysis

7. **Add 250 µl of FABG Buffer to the sample and mix by vortex.**
8. **Incubate for 30 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.**
9. **Preheat required Elution Buffer (for Step 6 DNA Elution) in a 70°C water bath.**
10. (Optional Step)**: If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.**

Step 4 – Binding

11. **Add 250µl ethanol (96~100%) to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate.)**

12. **Place a FABG Column to a 2ml collection tube. Transfer the sample mixture (including any precipitate) to FABG Column. Centrifuge for 5 minute at full speed (14,000 rpm or 10,000 x g) and discard the 2ml collection tube. Place the FABG Column in a new 2ml Collection tube.**

Step 5 – Washing

13. **Wash FABG Column with 400µl W1 Buffer. Centrifuge for 1 min at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.**

14. **Place the FABG Column back in the 2ml Collection tube. Wash FABG Column with 600µl Wash Buffer (ethanol added). Centrifuge for 1 min at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.**
    ––Make sure that ethanol has been added into Wash Buffer when first open.

15. **Place the FABG Column back in the 2ml Collection tube. Centrifuge for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column.**
    ––Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step 6 – Elution

16. **Place the dry FABG Column to a new 1.5ml microcentrifuge tube.**

17. **Add 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column.**
    ––Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely.

18. **Incubate the FAGB Column at 37°C for 10 minutes in an incubator.**

19. **Centrifuge for 1 minute at full speed (14,000 rpm or 10,000 x g) to elute the DNA .**
    ––Standard volume for elution is 100 **µ**l. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 **µ**l.

Step Final - Pure DNA

20. **Store the DNA fragment at 4°C or -20°C.**

Special Protocol: (For Cultured Cell)

Step 1-Sample Preparation

A. For Cultured Animal Cells:

i. Trypsinize the adherent cells before harvesting.

ii. Transfer the appropriate number of cell (up to 1 x 10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 6,000 x g for 20 seconds.

iii. Remove the supernatant and resuspend the cells with 150 µl of RBC Lysis Buffer. Then follow Step 2 (Cell Lysis).

B. For Fresh blood (except human blood):

i. The sample volume of mammalian blood (non-nucleated) can be up to 50µl; the sample volume of nucleated erythrocytes (eg. bird or fish) can be up to 10µl.

ii. Add 150 µl of FATG Buffer and the blood sample into a 1.5ml microcentrifuge tube (not provided). Mix by vortex and then follow Step 2 (Cell Lysis).

Step 2 –Cell Lysis

1. **Add 200 µl of FABG Buffer to the sample and vortex for 5 seconds.**

2. **Incubate for 10 minutes at 70°C or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.**

3. **Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.**

4. (Optional Step)**: If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.**

5. **Follow the General Protocol starting from Step 3 (Binding).**

Special Protocol: (For Bacteria)

Step 1-Sample Preparation

A. For Gram-negative bacteria:

i. Transfer the appropriate number of bacterial cell (up to 1 x 10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute. Then discard the supernatant.

ii. Add 200 µl of FATG Buffer and resuspend the pellet by vortex or pipetting. Incubate for 5 minutes at room temperature.

iii. Follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

B. For Gram-positive bacteria:

i. Transfer the appropriate number of bacterial cell (up to 1 x 10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute. Then discard the supernatant.

ii. Add 200 µl of lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100, pH 8.0; prepare fresh lysozyme buffer immediately prior to use) and resuspend the pellet by vortex or pipetting.

iii. Incubate for 10 minutes at room temperature. During incubation, invert the tube every 2-3 minutes.

iv. Follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

Special Protocol: (For Fungus)

Step 1-Sample Preparation

i. Harvest appropriate number of fungus cell (up to 5 x 10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 5,000 x g for 10 minute. Then discard the supernatant.

ii. Add 600 µl of sorbitol buffer (1.2 M sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35mM ß-mercaptoethanol) and resuspend the pellet.

iii. Add 200 U of lyticase or zymolase. Incubate for 30 minutes at 30°C.

iv. Centrifuge the mixture at 2,000 x g for 10 minutes to harvest the spheroplast, and then remove the supernatant.

v. Add 200 µl of FATG Buffer to the tube and resuspend the cell pellet by vortex or pipetting.

vi. Incubate at room temperature for 5 minutes, and then follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

Troubleshooting:

Low yield

• Too many cells were used
--reduce the sample volume.

• Poor cell lysis because of insufficient Proteinase K activity
--Use a fresh or well-stored Proteinase K stock solution.

• Poor cell lysis because of insufficient mixing with FABG buffer
--Mix the sample and FABG Buffer immediately and thoroughly by pulse-vortexing.

• Poor cell lysis because of insufficient incubation time
--Extend the incubation time and make sure that no residual particulates remain.

• Ethanol is not added into the lysate before transferring into FABG Midi Column

• Ethanol is not added into Wash Buffer when first open; the volume or the percentage of ethanol is not correct before adding into Wash Buffer.

• Elution of genomic DNA is not efficient
--Make sure the pH of ddH_2O is between 7.5- 8.5.
--After Elution Buffer or ddH_2O is added, stand the FABG Midi Column for 5-10 min before centrifugation.

Column is clogged

•Blood sample contains clots
--Mix the blood sample well with anti-coagulant to prevent formation of blood clots.

•Sample is too viscous
--Reduce the sample volume.

Purified DNA dose not perform well in downstream application

•Sample is old
--Always use fresh or well-stored sample for genomic DNA extraction.

•Residual ethanol contamination
--After Wash step, centrifuge at 4,000 x g for an additional 10 minutes to dry the FABG Midi Column.

•RNA contamination